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FALSE-NEGATIVE *HISTOPLASMA* ANTIGEN IN ACUTE PULMONARY HISTOPLASMOSIS:

THE VALUE OF URINARY CONCENTRATION BY ULTRAFILTRATION AND HEAT DENATURATION OF SERUM PROTEINS IN DETECTION OF HISTOPLASMA ANTIGEN

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Abstract

We report an infant with localized pulmonary histoplasmosis in whom *Histoplasma* antibody assays, quantitative *Histoplasma* urine and serum antigen concentrations, and histopathologic findings of a mediastinal mass were nondiagnostic. A provisional diagnosis of histoplasmosis was established by using laboratory methods that increase the sensitivity of the antigen assay using ultrafiltration of urine and ethylenediaminetetraacetic acid/heat denaturation of serum proteins.

Keywords

histoplasmosis; urinary; serum; antigen

Nonprogressive pulmonary histoplasmosis of infants is unusual and those affected often present with a mediastinal mass. Since neoplasm is a consideration, prompt diagnosis is essential. If seropositivity cannot be demonstrated, as commonly occurs in early infection and in progressively disseminating histoplasmosis, an invasive diagnostic procedure may be needed.[1] Since histoplasmosis is uniformly associated with primary hematogenous dissemination that is limited by the development of an effective cellular immune response, sensitive and specific quantitative assays of *Histoplasma* antigen have been shown to provide strong diagnostic support early in infection.[2] Recently, modification of the methodology to incorporate ultracentrifugation of urine samples, has been shown to substantially increase test sensitivity.[3] Ethylenediaminetetraacetic acid (EDTA) denaturation of serum specimens improves the sensitivity for detection of antigenemia (Wheat LJ, submitted for publication).

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We report a 5-month-old boy who presented with wheezing and was found to have a mediastinal mass. Histoplasmosis was suspected; however, antibody titers were nondiagnostic, but consistent with possible histoplasmosis; and standard *Histoplasma* antigen assays of serum and urine were negative. Urine and serum antigen were detected using urine ultrafiltration and heat denaturation of serum proteins. The diagnosis of histoplasmosis was later confirmed by antibody seroconversion.

CASE HISTORY

A 5-month-old previously well boy presented with increased work of breathing. His only previous illness had been an upper respiratory infection and possible bronchiolitis in the prior month. The family resided in Tennessee and the parents recalled that he had accompanied them on a hike in a state park. They reported no symptoms or illness in other family members.

On admission, his weight was 6.5 kg, respiratory rate was 65 breaths/min, heart rate was 115/min, and blood pressure was 86/62 mm Hg. Oxygen saturation was 96% in room air. Examination revealed a tachypneic, nontoxic infant with prominent intercostal chest retractions. There was no lymphadenopathy, pallor, or facial puffiness. The trachea was in the midline. Pertinent physical findings were diminished air entry in the right chest, audible expiratory wheezing, and no audible crackles. The cardiac examination was normal and there was no hepatosplenomegaly. The balance of the physical examination was normal.

A complete blood count showed a hemoglobin of 9.7 g/dL, white blood cell count of 18,600 cells/μL with 71% neutrophils, 25% lymphocytes, 2% monocytes, 1% eosinophils, 1% basophils, and a platelet count of 533,000/μL. The serum electrolytes were normal. Lactate dehydrogenase was 309 U/L. A chest radiograph showed deviation of the mediastinum to the left and hyperexpansion of the right middle and right lower lobes. There was no pleural effusion. Computerized tomography demonstrated a posterior mediastinal mass with an infiltrative process in the right upper lobe associated with right paratracheal and subcarinal lymphadenopathy. The carinal mass encased and compressed both the right main stem bronchus and right pulmonary artery. There was atelectasis of the right lower lobe. Bronchoscopy revealed external compression of the right mainstem bronchus. The differential diagnosis of the posterior and middle mediastinal mass included neurogenic tumors, lymphoma, teratoma, germ cell tumors, and infection, especially granulomatous infection due to histoplasmosis or tuberculosis. The tuberculin skin test was nonreactive and acid-fast bacilli were not seen in gastric aspirates. *Histoplasma* complement fixation (CF) titers to both yeast and mycelial antigens were nonreactive; *Histoplasma* immunodiffusion antibody titers demonstrated an M band. Standard *Histoplasma* antigen assays of both serum and urine were negative (MiraVista Diagnostics, Indianapolis, IN). Thereafter, an open biopsy of the subcarinal mass was performed and histopathologic findings included necrotizing granulomatous inflammation. Gomori methenamine silver, periodic acid-Schiff and acid-fast stains were negative and all cultures of the biopsy specimen were negative. HIV antibody was nonreactive. Since the diagnosis of histoplasmosis was still suspected, augmentation of the sensitivity of the *Histoplasma* antigen assay using ultrafiltration of urine and EDTA and heat denaturation of serum proteins was performed.

METHODS

Four milliliters of urine was centrifuged at 3656*g* for 16 minutes to yield a final volume of 0.4 mL (10-fold concentration). Thereafter, the retentate was tested in the MVista *Histoplasma* antigen enzyme immunoassay (MV EIA).[2] For quantitation, the antigen content of the assay calibrators was determined by comparison to reference standards containing known concentrations of *Histoplasma capsulatum* galactomannan. Samples with optical density values less than the cutoff for positivity were reported as "none detected." Specimens with optical density readings that were greater than or equal to the cutoff for positivity, but that were less than the 0.6 ng/mL standard, were reported as "positive, <0.6 ng/mL." Antigen assay of serum was performed on a 300 μL sample which was treated with 100 μL of EDTA at 104°C for 4 minutes. It was then tested in the MV EIA, as recommended in the Platelia *Aspergillus* antigen EIA.[2]

RESULTS

Results of the standard quantitative antigen assay performed on the nonconcentrated urine sample and the non-EDTA treated serum sample were negative. Repeat testing after ultracentrifugation of the urine specimen yielded a positive result, <0.6 ng/mL. The EDTA-treated serum sample yielded a positive result of 2.11 ng/mL.

The patient was treated with itraconazole, 5 mg/kg/d administered orally for 10 weeks. Prednisone, 2 mg/kg/d was administered orally for 1 week followed by a 1 week taper. The patient was discharged after 6 days in no respiratory distress. At follow-up 6 weeks later, he was thriving and had no respiratory symptoms. A repeat *Histoplasma* CF test showed a titer of 1:128 to the yeast antigen, thereby confirming the diagnosis of histoplasmosis.

DISCUSSION

Histoplasmosis is caused by *H. capsulatum*, a dimorphic fungus found in large areas of the Ohio and Mississippi River Valleys. Infections are common in these regions and a sustained outbreak involving 100,000 cases has been reported.[4] Infection results from inhalation of microconidia that become aerosolized when contaminated environmental sites are disturbed. Spores germinate in the lung where they undergo transformation to the parasitic yeast form. Ninety-five percent of infections are unrecognized or self-limited. Risk factors for more serious disease, often accompanied by progressive hematogenous fungal dissemination, include intense exposure, primary or acquired disorders of cellular immunity, and relative immaturity of immune function in otherwise normal infants.[5]

Localized infection associated with pulmonary infiltrates and hilar/mediastinal adenopathy is the most common presentation in symptomatic patients. However, localized pulmonary infection and bronchial compression during early infancy is rare.[6] The only other reported case was that of an 18-month-old in whom computerized tomography of the chest showed a large, left mediastinal mass compressing the left, main-stem bronchus. Histologic examination of the nodes obtained by thoracotomy showed caseating granulomas with yeast forms typical of *H. capsulatum*.

Although isolation of the organism from cultures provides the strongest confirmatory evidence for infection, cultures are negative in most patients with localized disease. Antigen detection is useful in making an early diagnosis of histoplasmosis before antibodies appear.[7] Using standard antigen methodology, the sensitivity of antigen detection is greater in urine than serum.[8] In some cases, the antigen may be present in the urine below the assay's detection limit. False-negative results are more likely in localized pulmonary histoplasmosis than in diffuse pulmonary or disseminated disease.[9] Ultrafiltration could improve the sensitivity as shown by Egan et al[3] Among 84 false-negative specimens, 62 (73.8%) were positive after ultrafiltration ($P < 0.001$) versus 2% of controls. Our patient had antigenuria below the detection limit of the standard quantitative MV EIA, which was positive at <0.6 ng/mL after ultracentrifugation of the urine. The antigen was also not detected in the non-EDTA treated serum specimen but was found to be positive at 2.11 ng/mL after EDTA treatment of the serum at 104°C. These results provided support for our decision to treat the patient for histoplasmosis. Demonstration of precipitin antibodies to the *H. capsulatum* M protein antigen further supported the diagnosis of histoplasmosis. The seroconversion of the yeast CF test on follow-up testing confirmed the diagnosis. The infant responded well to therapy with itraconazole. A brief course of prednisone was used to promptly relieve the bronchial obstruction caused by the subcarinal lymphadenitis.[10] He remains well, 4 months after diagnosis. Further studies confirming the value of ultrafiltration and serum treatment are needed.

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